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TWO TAXA WITHIN THE NORTH AFRICAN *LESTES VIRENS* COMPLEX (ZYGOPTERA: LESTIDAE)

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A study of *Lestes "virens"* in Algeria, based on SEM, size analysis, and molecular analysis of nuclear ribosomal DNA genes (18S, 5.8S) and spacers (ITS1 and 2), reveals the presence of two taxa that can be separated by the length and sequence of their ITS1 and their adult coloration, but not by molecular features in their 18S and 5.8S genes, the ITS2 spacer, and morphology. This contrasts with the *Enallagma cyathigerum*-group, where geographically defined morphological differences were unaccompanied by differences in ITS1 and ITS2. Previous ecological data had shown the first lestid to be a summer, and the second an autumnal reproducer. The red autumnal species is here named *Lestes numidicus* sp. n. (holotype ♂, allotype ♀: Algeria, Lac des Oiseaux, X-1993; deposited in IRSN, Brussels); the status of the green summer species is discussed. It probably corresponds to *L. virens*, but this is likely to be a hybrid taxon, resulting from the postglacial introgression of *L. numidicus* with a taxon invading from the East, via the Iberian Peninsula. *L. virens vestalis* from France is likely to be introgressed as well. In case this hypothesis is confirmed, the first junior synonym available, *L. marikovskii* (Belyshev) from Kazakhstan, applies to the taxon extending from Kazakhstan-Tajikistan to Central Europe.

INTRODUCTION

The taxon *Lestes virens* was originally described by CHARPENTIER (1825) as *Agrion virens*, with Lusitania (the present Portugal) as the terra typica. Subsequently, RAMBUR (1840) described *Lestes vestalis*, without explicit statement of a type locality. Because Rambur worked in Paris, and indicated the non-French and sometimes French origin of most other taxa dealt with in his book, most subsequent authors have accepted "environs of Paris" as the terra typica for *L. vestalis*, which was soon reduced to a subspecies of *L. virens*. Currently, and although some authors continue to doubt the

validity of *vestalis* (ASKEW, 1988), it is widely believed that *L. v. virens* occupies southern France, the Iberian Peninsula, the main West Mediterranean islands, and the Maghreb, with *L. virens vestalis* appearing in central and northern France, Italy, western and central Europe, and the Balkan (ASKEW, 1988; DUMONT, 1991). JÖDICKE (1997) gives a complete overview of the range of both subspecies, as currently understood. However, outside of Europe, *L. virens vestalis* definitely extends into Anatolia (HJD has specimens from southern and eastern Anatolia as far as the Caucasus), reaching deep into western Asia, including Tajikistan and Kazakhstan. The subspecific status of populations of the Levant was left undetermined by DUMONT (1991), but nearer to *vestalis* Auctorum than to *virens* s. s.; a re-examination of these specimens (from the Golan heights in Syria, and the Jordan valley in Israel) now confirms that they are "dark bronze green", and thus fall into the category "*vestalis*", although they have a reddish sheen that sets them apart from all other populations.

Recently, the rediscovery of the ♂ type (in fact two specimens) from Portugal in the Museum of Natural History in Berlin has led HARTUNG (1993; 1996) to suggest that typical populations are characterised by a two-coloured pterostigma (the apical third up to the white apical cross vein, is much paler than the basal two thirds of the pterostigma), and may be distinct from populations elsewhere in the Mediterranean.

This paper addresses the status of two distinguishable entities, both previously known as *Lestes virens virens,* in Algeria (SAMRAOUI & MENAI, 1999) and other parts of North Africa (JACQUEMIN & BOUDOT, 1999; JÖDICKE et al., 2000). These two taxa have recently been shown to be reproductively isolated by a different period of reproduction at the same water bodies (SAMRAOUI & CORBET, 2000, 2002). They thus conform to the definition of two biological species (MAYR, 1942, 1963). The present study uses molecular and morphological data to clarify their status.

MATERIAL AND METHODS

Material subjected to molecular analysis is from Numidia, northeastern Algeria. For morphological comparison, specimens from Tunisia, Morocco, Spain, France, the Netherlands, Switserland, Anatolia, Syria, Israel, and Tajikistan were examined (see list below), either under a stereomicroscope, or under a JSM-840 scanning electron microscope (SEM) at 15 kV. From five populations, a sufficient number of ♂s was available to make a size analysis (abdomen length). These five series were subjected to an ANOVA and to a paired, two-tailed heteroscedastic t-test (variances unequal). The null-hypothesis was that the means are equal ($m_1 = m_2$, thus $\Delta m = o$), and exact probabilities that the means of each of the pair-wise combinations were equal were calculated and tabulated (Tabs I-II).

MATERIAL OF THE *LESTES "VIRENS"* GROUP EXAMINED. – Algeria: Lac des Oiseaux, early summer taxon (*"virens"*) (VI-1993): 9 ♂, 4 ♀; late summer taxon (*numidicus* sp. n.) **holotype** ♂, **allotype** ♀: X-1993, deposited in IRSN, Brussels; paratypes: 17 ♂, 6 ♀, same locality and date; – Tunisia: Tabarca, 8-VI-1976, 1 ♂; – Morocco: Larache, 6-VIII-1971, 1 ♂; – Spain: Canete, 12-VII-1989, 1 ♂; – Espiel (Cordoba), Sierra Morena, 27-VII-1978, 3 ♂, 2 ♀ (M. Ferreras leg.); – Cano Madre de las Marismas, El Rocio, Donana national park (Huelva), 7-VII-1976, 1 ♂, 1 ♀ (M. Ferreras leg.); – Pinell de Broi (Tarragona, Catalunya), 1-VII-1992, 2 ♂, 2 ♀ (R. Jödicke leg.); – Mont-Roig del Camp (Tarragona), 28-X-1992, 1 ♂ with reddish sheens on sides of synthorax (R. Jödicke leg.); – France: Riou near Caves (Aude), 29-VIII-1993 (H. Kintler leg., in coll R. Jödicke), 1 ♂ with reddish sheens on

sides of synthorax; – Syria: Golan Heights (Birket Bab el Haoua), 26-VI-1972, 7 ♂, 2 ♀; – Israel: Hadera fishponds, 17-VI-1972, 1 ♂; – Netherlands: Grote Peel (Limburg), 7-IX-1969, 8 ♂, 2 ♀; – Switzerland: Bourgdorf, 18-VI-1967, 1 ♂, 1 ♀; – Turkey (Anatolia): Beyshehir lake, 25-VII-1987, 4 ♂, 2 ♀; – Swamp at foot of mount Ararat, 3 ♂; – Tajikistan: Garm, 21-VIII-1987, 16 ♂, 2 ♀; – Fayzabad, 20-VIII-1987, 10 ♂.

EXPERIMENTAL PROCEDURES. – Muscular tissue was isolated from the thorax and total DNA prepared according to the protocol of the Puregene™ DNA isolation kit, type D-5000A (BIOzym, Landgraaf, the Netherlands. The complete region of the ribosomal spacers (ITS1 and ITS2) and the ribosomal 18S, 5.8S and part of the 28S genes was amplified using the polymerase chain reaction (PCR) with Qiagen DNA polymerase (Westburg, Leusden, the Netherlands). Eukaryote-specific external primers complementary to the 5'-terminus of the small subunit ribosomal (18S rDNA) gene (5'-TYCCTGGTTGATYYTGCCAG-3') and the 5'-terminus of the large subunit ribosomal (28S rDNA) gene (5'-TCCTCCGCTTABTDATATGCTTAA-3') were used to amplify the entire 18S-ITS1-5.8S-ITS2 and part of the 28S region. PCR amplifications were done using a total volume of 100 µl, containing 1,5 mM $MgCl_2$, 0.5 µM each primer, 0.2 mM dNTP mixture, and 10x Taq polymerase reaction buffer, and 2.5 units of Taq DNA polymerase (Qiagen) was added to each reaction. The samples were covered with two drops of mineral oil, and PCR reactions were performed in a Progene thermal cycler (NBS-Techne). Cycling conditions were 95°C for 1 min, 52°C for 2 min, and 72°C for 3 min for 30 cycles. External (see above) and internal primers in conserved regions of the 18S rDNA; 570C, 570, 1262C and 1262 (WEEKERS et al., 1994), 3'-RV (5'-TGATCCATCTGCAGGTTYCACCT-3'), ITS-FW (5'-TAGAGGAAGTAAAAGTCG-3'), and in conserved regions of the 5.8S rDNA; 5.8SFW (5'-TGGATCACTCGGCTCGT-3'), 5.8SRV (5'-CTGCCATGTGCGTTCGAAG-3') were used for sequencing. PCR products were used for direct sequencing using the BigDye™ technology, the protocol of the ABI Prism BigDye terminator cycle sequencing ready reaction kit, and thereafter analysed on an ABI Prism 377 DNA sequencer (PE Applied Biosystems).

SEQUENCE DATA ALIGNMENT AND PHYLOGENETIC ANALYSIS. – Sequences were aligned automatically using CLUSTALW (THOMPSON et al., 1994), and the alignments were visually optimized using the ESEE program (the Eyeball Sequence Editor; CABOT, 1989). Two different datasets were used for phylogenetic analysis; one containing the 18S, ITS1, 5.8S, ITS2 and partial 28S sequence data, the second containing only the ITS1, 5.8S and ITS2 sequence data. Phylogenetic reconstructions were performed using the neighbor-joining method and the JIN & NEI (1990) correction method of Treecon 1.3b (VAN DE PEER & DE WACHTER, 1994); bootstrap values were calculated with the same program (FELSENSTEIN, 1985) to assess the stability of each branching point.

RESULTS

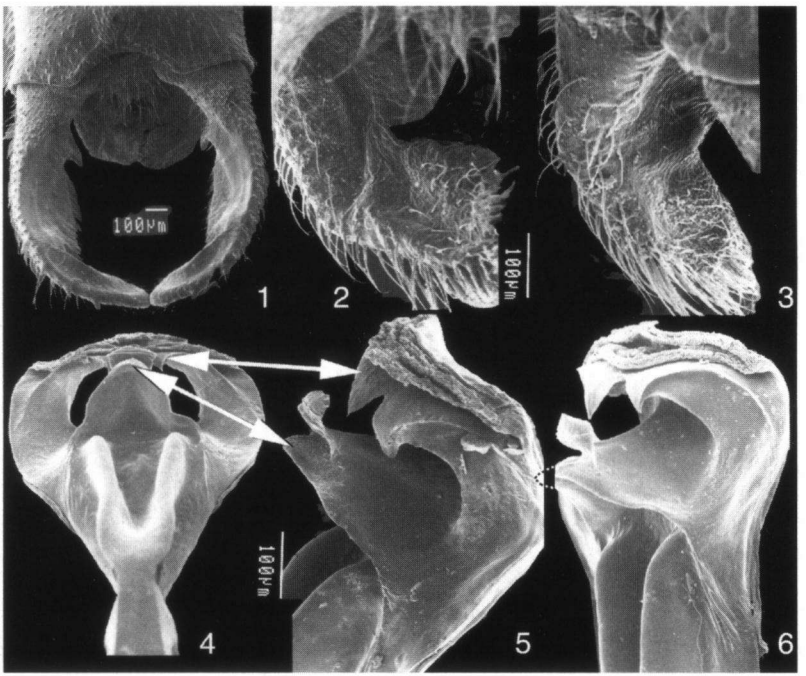
MORPHOLOGY

The "summer" taxon (reproducing mostly in July) had bright emerald green metallic colours, against a citron-yellow background. The yellow antehumeral stripes on the synthorax and the clear margins of abdominal segments 8-10 had narrow melanic borders. Rare specimens had a slight cupric metallic sheen. In contrast, the "late summer" taxon (reproducing mostly in September-October) had bright reddish metallic sheens against a deep ochraceous background. No specimens showed green tinges. Specimens from central Europe and the Asian specimens appeared deep emerald green, largely because the terminal segments and the antehumeral stripes of both sexes had broader melanin borders, sometimes constricting and even partly obliterating the antehumeral bands. Specimens from the Golan (but not the one ♂ from Hadera) were deep bronze-

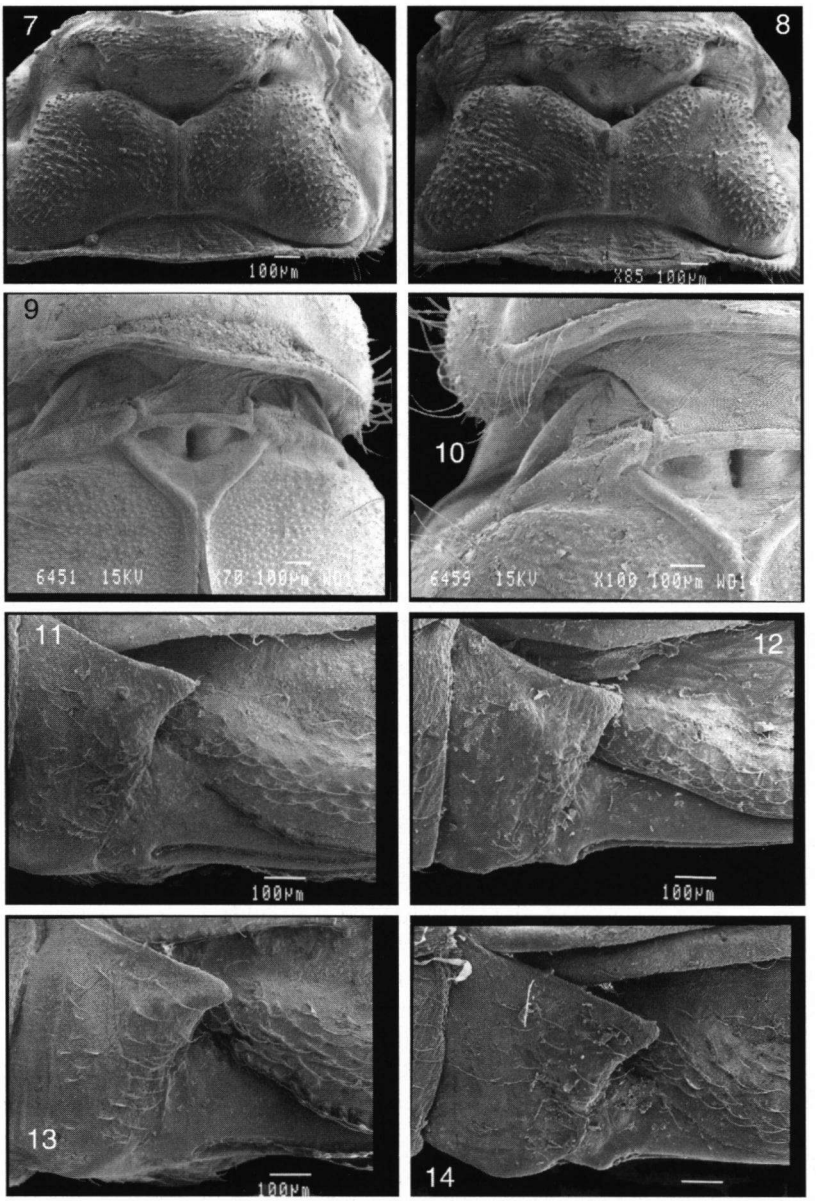
green, with a reddish sheen. Specimens from the Iberian peninsula, Morocco, and Tunisia were clear emerald green, like the early summer specimens from Lac des Oiseaux, except the specimens from Mont-Roig del Camp (Spain), and the specimen from Rio (Aude, France), which showed the reddish sheens typical of "late" specimens from Lac des Oiseaux.

We examined structural characters of numerous specimens of all groups for the following traits: shape of the superior and inferior ♂ appendages, ligula (males), and pronotum and ovipositor (females) (Figs 1-14). We failed to find any consistent difference, apart from occasional and individual teratologies.

Morphometry (Tabs I-II) revealed that specimens from West Asia were very significantly smaller than all others, except the strange series from the Golan heights, which was at the limit of significance. The early and late summer populations from Lac des Oiseaux in Numidia were clearly of the same size, and the Dutch population, could not be differentiated size-wise from either of them, although there is some evidence for a size gradient.



Figs 1-6. SEM's of males of the *Lestes "virens"*-group from Lac des Oiseaux, Numidia, Algeria: (1) terminalia of an early summer male; – (2) golf-club shaped appendix inferior of an early summer male; – (3) same, of a late summer male (*numidicus* sp. n.); – (4) ligula, dorsal view; – (5) same of an early summer male, lateral view; – (6) same of a late summer male.



Figs 7-14. SEM's of females of the *Lestes "virens"*-group from Lac des Oiseaux, Numidia, Algeria: (7) pronotum of an early summer female; – (8) same of a late summer (*numidicus* sp. n.) female; – (9) carinal fork and lamina mesostigmalis of an early summer female; – (10) same of a late summer female, somewhat more enlarged; – (11, 13) two shapes of valvifers in early summer females; – (12, 14) same in late summer females.

Table I
Basic statistics

Groups	N	Sum	Average	Variance Range
Grote Peel	8	232	29	0.357
Faizabad	16	411	25.68	0.79
Lac Oiseaux X	10	292	29.2	1.06
Golan heights	7	188	26.85	1.72
Lac Oiseaux VI	7	206.5	29.5	2.75

ANOVA

Source of variation	SS	df	MS	F	F critical	P-value
Between groups	128.47	4	32.11	27.13	3.78	< 0.001
Within groups	50.89	43	1.18			
Total	179.36	47				

DNA STUDY

We obtained the complete, unambiguous sequence for the ribosomal spacers (ITS1 and ITS2) and the ribosomal 18S, 5.8S and partial 28S rDNA genes of *Lestes* "early summer", *Lestes* "late summer", *Lestes macrostigma, Chalcolestes viridis* (syn: *Lestes viridis*) and *Sympecma fusca* (outgroup). The lengths of the coding genes (18S, 5.8S), the internal transcribed spacers (ITS1, ITS2), as well as their G+C content are listed in Table III. The 18S and 5.8S gene of Lestes "early summer", *Lestes* "late summer" and *L. macrostigma* are identical, and therefore phylogenetically not informative. The internal transcribed spacers (ITS1 and 2) do contain phylogenetically informative signals for these tree taxa (8,11% sequence variation). The ITS1 is highly variable and contains 21 base pair differences (9 indels and 12 substitutions) between the two "*virens*" members, and 48 including *L. macrostigma*. The ITS2 contains far less variation: for the two "*virens*" members it is identical, and if compared to *L. macrostigma* there are only 12 differences (2 indels and 10 substitutions). The sequence variation among the two "*virens*" members and *L. macrostigma* are shown in Figure 1. The sequence variation between Lestes "early summer", *Lestes* "late summer" is 2.85% for both ITS1 and ITS2 regions together. However, since the ITS2 region contains no sequence variation at all (0%), the real amount of sequence variation in the ITS1 region is 7.81%. The sequence variation is caused by 9 indels and 12 substitutions in 13 regions of the ITS1 (Fig. 15).

Phylogenetic analysis, using the method described above, resulted in trees, inferred from evolutionary distances in a distance matrix method. The phylogenetic trees for the two different datasets, 18S-ITS1-5.8S-ITS2-28S region (tree not shown), and ITS1-5.8S-ITS2 region (Fig. 16) resulted in identical tree topologies with high bootstrap support. Although showing some genetic distance, the two "*virens*"-like taxa cluster

Table II

Probability values for paired t-tests under the null hypothesis that the means of the ♂ abdomen lengths in the five groups are identical. Values for which the null hypothesis is not supported (=the means are different) are underlined

P-values	Grote peel	Faizabad	Lac Oiseaux X	Golan Heights	Lac Oiseaux V I
Grote peel	-	< 0.0001	0.44	0.002	0.47
Faizabad	< 0.0001	-	< 0.0001	0.06	< 0.0001
Lac Oiseaux X	0.44	< 0.0001	-	0.002	0.68
Golan heights	0.0053	0.06	0.002	-	0.007
Lac Oiseaux VI	0.38	0.0004	0.68	0.007	-

together and are clearly derived from a common ancestor (Tab. IV). They are closer related to *L. macrostigma* than to *C. viridis* (that is, incidentally, showing such a large distance from true *Lestes* that the status of a separate genus seems well justified) and *S. fusca* .

DISCUSSION

Lestes "virens" was previously divided into the nominal taxon, *L. v. virens* (Charp.), and *L. virens vestalis* Rambur. The specimens from the East here examined conform to what dragonfly students currently equate with *L. virens vestalis;* Iberian and Maghrebian specimens, in contrast, should be *L. v. virens*. However, if two taxa, separated by mating season and differing in ITS1 sequence live in Algeria, which of them is the true *virens*?

The rediscovery by HARTUNG (1993) of the type of *Lestes virens* (CHARPENTIER, 1825) in the Museum of Natural History (Museum Berolinum) in Berlin was helpful in solving this question, but it also added a new complication, since the "true" *L. virens* with bicoloured pterostigma seems primarily to inhabit the more humid west coast of Iberia. The "two-colours" condition of these populations clearly deserves more study, but might be a local condition without taxonomic significance, although this remains to be tested (see below). The brown pterostigma of *L. virens* is set between cross veins that are white and bordered by a diffuse white zone, which in extreme cases creates a "two-colour" condition. Perhaps the emerald green colour of the types is more relevant, and suggests it is the same taxon as the "early summer" animals of the El Kala wetlands. The "late" specimens from Spain and France have the same cupric sheen as the late specimens of Numidia, suggesting that the two taxa might still extend to the Iberian peninsula and even the South of France.

Regarding the status of the two "*virens*-like" taxa, it appears reasonable to consider both as products of evolution that have not yet reached reproductive isolation, yet are sufficiently distinct at the molecular and ecological levels to be ranked as species.

We here offer the following hypothesis, which appears to be the most parsimonious: the taxon living in the Iberian Peninsula and in the Maghreb (with "short" ITS1),

Table III
Gene length, GC content and accession number of the ribosomal 18S and 5.8S genes and the ITS1 and ITS2 spacers, and geographical origin of the species used in this study. Collectors' institutional affiliations are provided in the Acknowledgments

Species	18S gene		ITS-1 spacer		5.8S gene		ITS-2 spacer		EMBL acc. number	Geographical origin	Collector
	length	GC %	length	GC %	length	GC %	length	GC %			
L "virens"	1866	51.07	260	47.69	179	54.75	291	70.45	AJ421951	Lac Oiseaux, Algeria	B. Samraoui
L. numidicus	1866	51.07	269	49.81	179	54.75	291	70.45	AJ421952	Lac Oiseaux, Algeria	B. Samraoui
L. macrostigma	1866	51.07	257	45.53	179	54.75	293	70.31	AJ421950	Anatolia, Turkey	M. Pavesi
C. viridis	1867	50.88	195	47.69	179	54.75	193	67.88	AJ421949	Ifrane, Morocco	H.J. Dumont
S. fusca	1866	50.91	256	48.83	179	54.75	267	63.67	AJ421948	Syr Darja, Uzbekistan	I. Mirabdulaev

reproducing relatively early in the season, is what Charpentier described as *Agrion virens*. However, it is not a pure species. Taxa, including dragonflies, that had previously been sejunct were forced in disjunction for tens of thousands years by the Würm and earlier glaciations. When glaciers last retreated around 18,000 BP, there was a re-advance of species into continental Europe from two major refugia: the Ibero-Magrebian in the West, and the Anatolian-West Asian in the East. For reasons, which are partly linked to topography, and partly unclear, eastern relicts often advanced quicker into Europe than western ones (HEWITT, 2000). In lestids, as in several other damselfly species like *Calopteryx splendens* and *C. xanthostoma* (WEEKERS et al., 2001), and in *Enallagma cyathigerum* and its subspecies (SAMRAOUI et al., 2002), disjunction initiated allopatric evolution. In some taxa this resulted in the appearance of morphological differences, in others not. The genetic distance achieved was, however, generally insufficient to prevent mixing between the western and eastern invaders: both groups could still interbreed. They gave rise to hybrid populations, currently still in the process of fusing. In *Lestes "virens"*, it seems that the western (red and clear) group reproduced later (and for a longer period: June-October) than the eastern (green and melanic) one (reproducing in June-July). Thus, the western gene pool partly fused with the eastern one, but the part of the western gene pool reproducing latest in the season remained unaffected by introgression. It can be distinguished on sight by its reddish metallic colours, a character shared by all specimens. In the "early reproducers", rare specimens may show a slight reddish sheen, but the reverse (late specimens showing green sheens and melanism) has not been seen so far. This is consistent with the hybrid nature of West European populations, which should show a genetic gradient and occasional specimen with parental colours from the southwest to the northeast.

It follows that both Charpentier and Rambur gave

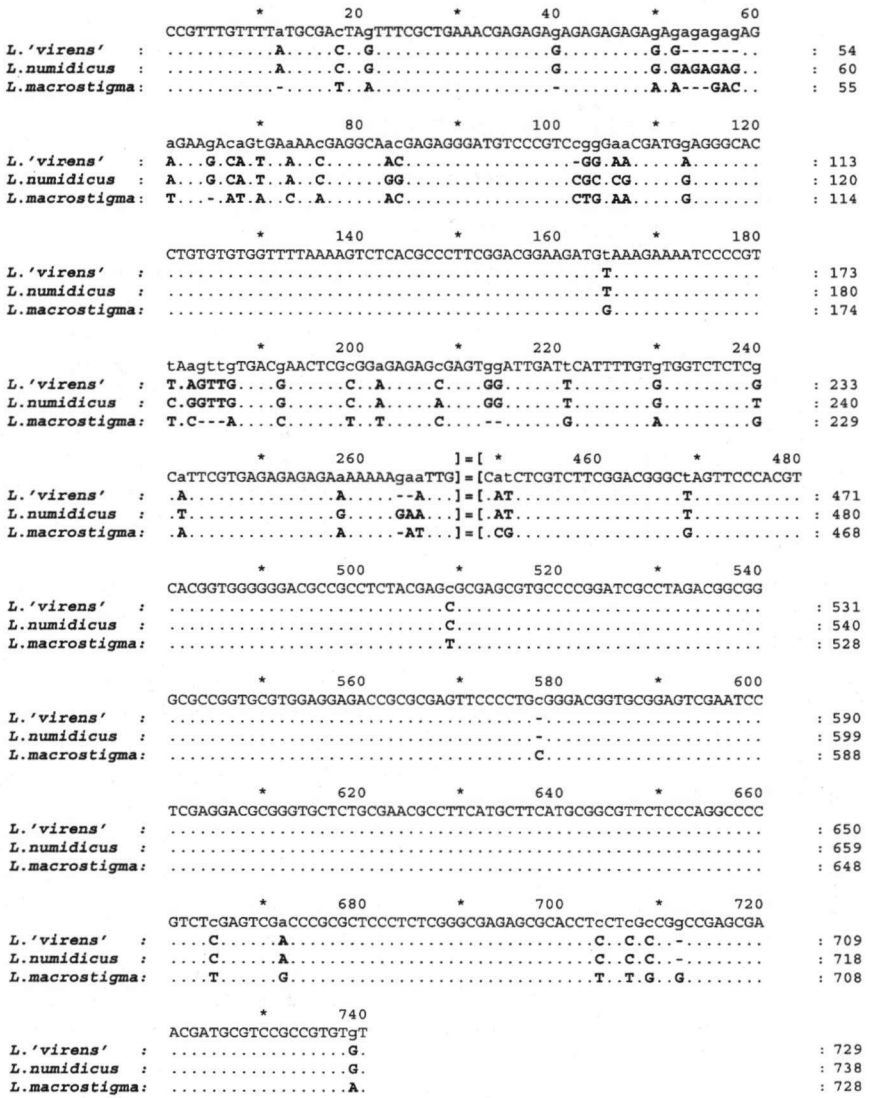


Fig. 15. Position of the variable sites in the hypervariable rDNA regions (ITS1 and ITS2) of *Lestes "virens"*, *L. numidicus* and *L. macrostigma*. The sequence alignment for the 5.8S gene region is excluded since all sites were identical;]=[indicates the position where the 5.8S gene is deleted. Bases 1 to 269 represent the ITS1 region; bases 449 to 740 represent the ITS2 region. The consensus sequence is shown on top and a number and an asterisk indicate the location in the ITS1-5.8S-ITS2 region.

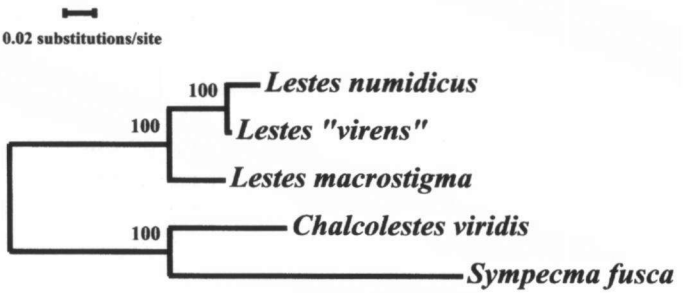


Fig. 16. Distance tree using the neighbor-joining method to show the phylogenetic position of the *Lestes "virens"*-group and *L. macrostigma*. The tree is rooted using *Chalcolestes viridis* and *Sympecma fusca* as outgroup. Numbers at the nodes give the number of times a cluster appeared in the consensus tree after a sequential bootstrap analysis of 100 runs.

scientific names to hybrid populations; such names are invalid. The "late" taxon of Numidia, conversely needs a name. We here name it *Lestes numidicus* sp. n., with the same morphology as "classical" *virens* but characterised by its strong cupric-red metallic sheens, and by the ribosomal gene sequence specified earlier. The holotype and allotype have been selected and deposited at the Royal Institute of Natural Sciences, Brussels, and a series of paratypes are in the collection of HJD. The eastern taxon also needs to be renamed, since *vestalis* is suspect. The first junior synonym available is *Lestes marikovskii* (BELYSHEV, 1961), from Kazakhstan. The description is brief, and states that the taxon (described as a subspecies) is structurally typical (= *virens*), but smaller. This appears sufficient to characterise all populations between central Asia and central Europe. The zone between central Europe and the Maghreb, especially as far as "early"

Table IV

Pairwise comparison of all in- and outgroup taxa in the analysis. Above the diagonal are absolute nucleotide differences. Below the diagonal are distances calculated using the Kimura 2-parameter parameter model as correction method. Values were calculated for the dataset containing only ITS1 and ITS2 regions (bold), and for the dataset containing the combined 18S, ITS1, 5.8S and ITS2 regions (brackets)

Species	*L. "viridis"*	*L. numidicus*	*L. macrostigma*	*C. viridis*	*S. fusca*
L. "virens"	—	**11**	**31**	**81**	**139**
	—	(11)	(31)	(87)	(148)
L. numidicus	**0.0201**	—	**41**	**85**	**143**
	(0.0042)	—	(41)	(91)	(152)
L. macrostigma	**0.0587**	**0.0781**	—	**80**	**137**
	(0.0120)	(0.0159)	—	(86)	(146)
C. viridis	**0.2588**	**0.2719**	**0.2569**	—	**71**
	(0.0367)	(0.0384)	(0.0363)	—	(80)
S. fusca	**0.3481**	**0.3582**	**0.3432**	**0.2118**	—
	(0.0603)	(0.0619)	(0.0595)	(0.0335)	—

populations are concerned, is to be declared a hybrid zone, however. The population from the Golan Heights in Syria remains hard to place, and clearly deserves to be included in any future study. It is not to be excluded a priori that the Levantine populations had a brief period of contact with the Magrebian ones, through Egypt and Libya.

The advantage of this hypothesis is that it is open to testing by molecular methods: what is needed is a series of ITS1 sequences of populations situated between central Asia, western Europe, and the Maghreb. The differences in gene length and substitution rates should enable us to learn more about the dynamics of the introgression event.

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REFERENCES

ASKEW, R.R., 1988. *The dragonflies of Europe*. Harley Books, Colchester

BELYSHEV, B.F., 1961. Notes on the fauna of Odonata of eastern Kazakhstan. *Fragm. faun.* 9: 27-42. – [in Russ.]

CABOT, E.L. & A.T. BECKENBACH, 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. *Comput. Appl. Biosci.* 55: 233-234.

CHARPENTIER, T. de , 1825. *Horae entomologicae*. Gosohorsky, Wratislaviae.

DUMONT, H.J., 1991. *Odonata of the Levant. [Fauna Palaestina, Insecta, 5].* Israel Acad. Sci. Human., Jerusalem.

FELSENSTEIN, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

HARTUNG, M., 1993. *What is the true Lestes virens virens (Charpentier, 1825)?* Poster 12th int. Symp. Odonatol., Osaka.

HARTUNG, M., 1996. Odonata from the Iberian Peninsula with a description of Calopteryx haemorrhoidalis almogravensis ssp. n. from Portugal. Studies on Iberian dragonflies. *Adv. Odonatol.* (Suppl.) 1: 53-59.

HEWITT, G., 2000. The genetic legacy of the Quaternary ice ages. *Nature, Lond.* 405: 907-913.

JACQUEMIN, G. & J.P. BOUDOT, 1999. *Les libellules (odonates) du Maroc*. Soc. Fr. Odonatol., Bois d'Arcy.

JIN, L. & M. NEI, 1990. Limitations of the evolutionary parsimony method of phylogenetic analysis. *Mol. Biol. Evol.* 7: 82-102.

JÖDICKE, R., 1997. *Die Binsenjungfern und Winterlibellen Europas, Lestidae*. Westarp, Magdeburg & Spektrum, Heidelberg.

JÖDICKE, R., J. ARLT, B. KUNZ, W. LOPAU & R. SEIDENBUSCH, 2000. The Odonata of Tunisia. *Int. J. Odonatol.* 3: 41-71.

MAYR, E., 1942. *Systematics and the origin of species*. Columbia Univ. Press, New York.

MAYR, E., 1963. *Animal species and evolution*. Harvard Univ. Press, Cambridge/MA.

RAMBUR, J.P., 1840. *Histoire naturelle des insectes neuroptères*. Roret, Paris.

SAMRAOUI, B. & P.S. CORBET, 2000. The dragonflies of Numidia, 2: seasonal ecology. *Int. J. Odonatol.*

3: 27-39.

SAMRAOUI, B. & P.S. CORBET, 2003. Seasonal ecology of Algerian Lestidae (Odonata: Zygoptera). *Int. J. Odonatol.* (submitted).

SAMRAOUI, B. & R. MENAÏ, 1999. A contribution to the study of Algerian Odonata. *Int. J. Odonatol.* 2: 145-165.

SAMRAOUI, B., P.H.H. WEEKERS & H.J. DUMONT, 2002. The Enallagma of the western and central Palaearctic (Zygoptera: Coenagrionidae). *Odonatologica* 31: 371-381.

THOMPSON, J.D., HIGGINS, D.G. & T.J. GIBSON, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucl. Acids Res.* 22: 4673-80.

VAN DE PEER, Y. & R. DE WACHTER, 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput. Appl. Biosci.* 10: 569-570.

WEEKERS, P.H.H., J.F. DE JONCKHEERE & H.J. DUMONT, 2001. Phylogenetic relationships inferred from ribosomal ITS sequences and biogeographic patterns in representatives of the genus Calopteryx (Insecta: Odonata) of the West Mediterranean and adjacent West European zone. *Mol. Phylog. Evol.* 20: 89-99.

WEEKERS, P.H.H., R.J. GAST, P.A. FUERST & T.J. BYERS, 1994. Sequence variations in small-subunit ribosomal RNAs of Hartmanella vermiformis and their phylogenetic implications. *Mol. Biol. Evol.* 11: 684-690.